MATERNAL PRE-PREGNANCY BMI, GESTATIONAL WEIGHT GAIN AND THE INFANT GUT MICROBIOME

by

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Abstract

Background

The intergenerational cycle of obesity, perpetuated by the continued rise of maternal obesity in the US, may be partly explained by mother-to-infant vertical transmission of microbiota. Prospective data to test this hypothesis are, however, still sparse.

Aim

To prospectively analyze maternal pre-pregnancy BMI and gestational weight gain in relation to the infant gut microbiome at 6 weeks in the New Hampshire Birth Cohort.

Methods

We ascertained exposure data from questionnaires and medical records. Infant gut microbiome data was generated from 6-week infant stool using Illumina 16S rRNA gene sequencing (V4-V5 region). Linear regression was used for microbial alpha-diversity models, permutational multivariate analysis of variance (PERMANOVA) for microbial beta-diversity (overall microbial community composition) models, zero inflated beta regression for differential abundance of the most abundant genera, and negative-binomial regression, with log-transformed microbial taxa, for differential abundance of operational taxonomic units (OTUs) models. Analyses were conducted before and after adjusting for potential confounders, which included maternal age, education, parity, and Mediterranean diet score. Informed by prior literature, analyses were conducted stratified by delivery mode. Sensitivity analyses were conducted via stratifying by infant sex and controlling for infant feeding.

Results

Among 335 mother-infant pairs, 56% had normal pre-pregnancy BMI (<25, referent), 27% were overweight (BMI 25.1-30), and 18% obese (BMI >30). Among 312 pairs with weight gain data, 10% had inadequate weight gain, 30% adequate (referent), and 60% excess (informed by the Institute of Medicine's recommended rates of weight gain during the second and third trimesters of pregnancy: 0.8 – 1 lb/wk for normal weight mothers, 0.5 – 0.7 lb/wk for overweight mothers, and 0.4 – 0.6 lb/wk for obese mothers). In the vaginal delivery strata, maternal obesity was independently associated with infant gut microbiome beta diversity (measured by Unweighted UniFrac), and higher infant gut microbiome alpha diversity (measured by number of observed species). In the vaginal delivery group, 15 operational taxonomic units (OTUs) were differentially abundant in the fully adjusted model (p <0.05 and FDR adjusted p < 0.1). Specifically, among infants born to overweight mothers there was overrepresentation of OTUs in the genera *Staphylococcus* and *Enterococcus*, and the species *Escherichia coli*, *Bacteroides fragilis*, and *Veillonella dispar*. Furthermore, monotonic linear graded associations between higher pre-pregnancy BMI and higher genera *Parabacteroides* and *Staphylococcus*, and lower *Bacteroides* were found in the vaginal delivery strata; the opposite trend was observed for *Bacteroides* in the Cesarean section strata (p-for interaction = 0.05). Importantly, there were no significant associations between pre-pregnancy BMI, or gestational weight gain categories with infant microbiome alpha diversity or OTUs among Cesarean section infants.

Conclusion

Maternal pre-pregnancy BMI and gestational weight gain may be associated with the infant gut microbiome diversity and composition at 6 weeks in vaginally delivered infants, but similar associations were not observed in Cesarean section delivered infants. These findings suggest

delivery-mode specific associations between maternal weight status and the infant microbiome. Furthermore, they suggest that the infant gut microbiome may play a role in the intergenerational transfer of obesity, which may inform delivery mode practices, and tactics for early life prevention of obesity.

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Preface

In August 2016, I matriculated to the Johns Hopkins Bloomberg School of Public Health in Baltimore, MD to pursue a Master of Health Science degree in Epidemiology, in the Cardiovascular Disease and Clinical Epidemiology track. I have since been advised by Dr. Noel T. Mueller, Assistant Professor at the Welch Center for Prevention, Epidemiology and Clinical Research in the Department of Epidemiology.

Motivated by an interest to understand the mechanisms behind the intergenerational cycle of obesity, between mother and her child, my thesis project aims to analyze the associations between maternal pre-pregnancy BMI, gestational weight gain, and the infant gut microbiome at 6 weeks of age. This study was conducted in the New Hampshire Birth Cohort with the support of Dr. Margaret Karagas and colleagues from the Department of Epidemiology at the Dartmouth Geisel School of Medicine.

Acknowledgements

I would like to begin by extending my deepest gratitude to Dr. Noel Mueller, who has gone above and beyond as an advisor, and mentor since I began working with him in the summer of 2016. At the time, I was thrilled at the prospect of working on a paper fresh out of my undergraduate program, and was honored to be treated as a fellow researcher by Dr. Mueller. Throughout my time working with Noel, I was continuously humbled by his perseverance in supporting me as his student, and in conducting rigorous public health research that aims to have a substantial impact on the world. His continued patience and dedication in me as a mentee, belief in me as a capable researcher, and unrelenting positive energy has truly been transformative in encouraging me to continue pursuing public health research. I would also like to thank my wonderful mentors from the Department of Epidemiology at the Dartmouth Geisel School of Medicine, including Dr. Margaret Karagas, Dr. Juliet Madan, Dr. Modupe Coker, and Dr. Anne Hoen. Through their expertise, and continuous guidance throughout the process of this thesis, the final project was elevated above and beyond my initial expectations. I am also extremely thankful for the extensive help of Michael S. Zens, and John Hudson, who alongside Dr. Coker provided me with all the tools I needed to be successful in my analysis. Without their unending patience and support throughout the process of creating this thesis, I would have spent several more years on this project alone. Lastly, I would like to thank my fellow JHU students who became a family away from home here in Baltimore. Working alongside these kindhearted, public health driven students, and the world-renowned professors at Hopkins has fostered a great love for public health within me.

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Introduction

Overweight and obesity (OWOB), particularly during childhood, continues to be of major public health concern due to its unrelenting rise in prevalence in the United States[1,2] and throughout the world[3]. Among children under 5, the global prevalence of OWOB rose from 30 million in 2000 to 41 million by 2016, with an additional 240 million OWOB children aged 5 – 19 years in 2016[4,5]. The global rise in OWOB, along with the intractable challenge of achieving sustained weight loss in OWOB individuals later in life, has led to an increased interest in the early-life origins of OWOB.

An important area of research on the early origins OWOB aims to elucidate, and eventually intervene upon, the mechanisms underpinning the intergenerational cycle of obesity between a mother and her child. Mothers that are OWOB are two to four times more likely to give birth to children who will become OWOB, starting as early as 2 to 5 years of age[6-8]. Yet genome wide association studies have found that genetic polymorphisms likely only explain a small fraction of the heritability of OWOB[9]. One potential, yet understudied pathway that may help explain the intergenerational cycle involves the mother-to-newborn transmission of microbiota.

Recent advances in genome sequencing has led to the discovery and exploration of the vast human microbiome (the collection of microbial genomes in and on our bodies), which comprises > 8 million protein-encoding genes, approximately 360 times that of the human genome[10,11]. Because the microbiome is seeded at birth, it has been postulated that differential mother-to-newborn transfer of microbiota perpetuates the intergenerational cycle of obesity[12]. In our recent literature review on this topic, we concluded that there is a dearth of knowledge with

respect to the effects of maternal pre-pregnancy BMI and gestational weight gain on the infant microbiome, with conflicting results from the few studies on the subject[13]. Furthermore, we noted a lack of consistency in the statistical and microbial profiling methods employed by researchers in the field, along with small sample sizes and a lack of adjustment for potential confounders and evaluation of effect measure modifiers in these studies.

To address these gaps in the literature and to further elucidate the role of the microbiome in the intergenerational cycle of obesity, in the current study we aimed to rigorously examine the associations of pre-pregnancy BMI and gestational weight gain with the infant gut microbiome structure, diversity and composition at 6 weeks of age in the New Hampshire Birth Cohort. Importantly, we conducted analyses stratified by delivery mode, as this variable has been found to be a strong predictor of the infant gut microbiome in this cohort, among others, and it has been implicated as a potential modifier of the associations between pre-pregnancy BMI and the infant gut microbiome[14-16]. Consistent with the notion of mother-to-newborn vertical transmission of bacteria at birth, in particular *Bacteroides* spp.[17,18], and with our prior observations [15], we hypothesized that in vaginally-delivered infants maternal pre-pregnancy overweight and obesity (vs. normal weight) is associated with altered infant gut microbiota structure, diversity and composition.

Methods

Study Population

Detailed information on the New Hampshire Birth Cohort has been described previously[14,19]. Briefly, in 2009, women who were 18 to 45 years of age and between 24 and 28 weeks of gestation were recruited for participation in an observational birth cohort study. Mothers and their infants were then followed up at 6 weeks post-delivery. This timing was chosen due to its alignment with an established 6-week post-partum visit, and because infant feeding practices (breastfeeding v. formula feeding) are often well established by this age. During this visit, infant stools were collected for microbial community profiling. Data on covariates of interest were also collected using a combination of medical records, questionnaires, and telephone-calls.

Pre-pregnancy BMI was derived from self-reported weight and height from a prenatal questionnaire. Gestational weight gain information was ascertained from a postpartum questionnaire. We excluded 2 women missing pre-pregnancy BMI data, and 8 women with a pre-pregnancy BMI < 18.5 kg/m^2 (flowchart displayed in **Figure S1**). We then categorized the remaining 335 women as normal weight (18.5 kg/m^2 ≤ BMI < 25 kg/m^2), overweight (25 kg/m2 ≤ BMI < 30 kg/m^2), or obese (BMI ≥ 30 kg/m^2). For gestational weight gain, we categorized the 312 women who also had weight gain data as having experienced inadequate, adequate, or excess weight gain based on Gilmore and Redman's method[20], which relies on the Institute of Medicine's recommended rates of weight gain specific to their pre-pregnancy BMI category[21]. The recommendations state that during the second and third trimesters of pregnancy, normal

weight mothers are expected to gain 0.8 – 1 lb/wk, overweight mothers 0.5 – 0.7 lb/wk, and obese mothers 0.4 – 0.6 lb/week.

Microbial Sampling and 16s rRNA Sequencing

Infant stools were collected from diapers and aliquoted within 24 hours into sterile tubes and stored at -80 °C. DNA extraction was then conducted using Zymo DNA extraction kits on thawed samples, followed by quantity and purity assessment using OD260/280 nanodrop measurement[22]. Fusion primers were used to obtain 16S ribosomal RNA (rRNA) V4-V5 amplicons. One of eight five-nucleotide long forward primers was used to connect the Illumina-specific bridge and sequencing primer regions with the 16S region, whereas the reverse primer was simply one of 12 Illumina indices. 16S rRNA amplification of the V4-V5 hypervariable regions was then conducted at the Marine Biological Laboratory in Woods Hole, Massachusetts for microbial profiling using both forward and reverse primers[23,24]. Due to the use of both forward and reverse primers, 96 samples were multiplexed per lane. PCR was then conducted using triplicate samples of 33 μL, and a combination of 1.0 U Platinum Taq Hi-Fidelity Polymerase (Life Technologies), 1X Hi-Fidelity buffer, 200 μM dNTP3 PurePeak DNA polymerase mix (Pierce Nucleic Acid Technologies), 1.5 mM MgSO4 and 0.2 μM of each primer. Lastly, qPCR was used to quantify each library pool before sequencing using paired-end Illumina MiSeq 100 cycle runs.

Analytical Methods

Forward and reverse reads derived from Illumina sequencing were assembled and demultiplexed using CASAVA 1.8.2, and denoised and quality filtered using DADA2 in Quantitative Insights Into Microbial Ecology 2 (QIIME 2) version 2017.10 [25,26]. We then

employed QIIME 2 to assign operational taxonomic units (OTUs) using the Greengenes database with a 99% cutoff for similarity before importing the data into R 3.4.2 using the biom-format package in Python[27-30].

Unweighted UniFrac, weighted UniFrac, and Bray-Curtis distances were used to analyze microbial beta diversity (overall microbial community composition) after rarefying to a sampling depth of 1029 counts[31,32]. Principal coordinate analysis (PCoA) plots were created within QIIME 2 using Emperor[33]. We tested for differences in beta diversity using permutational multivariate analysis of variance (PERMANOVA) in QIIME 2 with 999 permutations. We also conducted PERMANOVA tests with 10,000 permutations, after adjusting for covariates that were potential confounders, using the vegan package in R[34]. We considered a p-value < 0.05 as statistically significant for PERMANOVA tests of beta diversity.

Microbial alpha diversity was estimated with observed OTUs (unique number of OTUs), Shannon diversity index, and Chao1 using the phyloseq package in R[29]. Data were rarefied to the same sampling depth of 1029, with 10,000 permutations. The associations of pre-pregnancy BMI and gestational weight gain with gut microbiota alpha diversity metrics were analyzed using linear regression. We considered a p-value < 0.05 as statistically significant for alpha diversity analyses.

We next compared the relative abundance of the most dominant genera (those > 1.0% in prevalence) in the infants' gut microbiota using zero-inflated beta regression in the gamlss package within R, which accounts for excessive zero counts[35]. Based on previous publications[15,18], we *a priori* hypothesized an association of maternal pre-pregnancy BMI categories with *Bacteroides* genus in the vaginal strata. With the DESeq2 package in R, we then agnostically assessed differential relative abundance of specific OTUs using a log-transformed

negative binomial model with an internal adjustment for library size[36]. To account for multiple-testing in this agnostic approach, we considered a False-Discovery-Rate (FDR) adjusted p-value < 0.10 as statistically meaningful. We did not rarefy OTU counts in differential abundance analyses because we wanted to maximize use of the sequencing data available[37].

We performed all regression analyses before and after stratifying by delivery mode, our *a priori* hypothesized effect measure modifier, as well as before and after adjusting for potential confounders. We adjusted for covariates that have been associated with our exposures (pre-pregnancy BMI and gestational weight gain) and our outcome (infant gut microbiome), but that are not on the causal pathway between these two, to avoid over adjusting our model for potential mediators. The final multivariable model included maternal age, parity, maternal educational achievement at time of pregnancy, and maternal Mediterranean diet score, excluding alcohol, during pregnancy.

We also conducted sensitivity analyses by further adjusting for feeding method at 6 weeks (defined as exclusively breastfed vs. combination fed or exclusively formula fed) and infant sex, as well as by restricting to infants exclusively breastfed at 6 weeks.

Finally, in addition to our *a priori* delivery-mode specific examination of associations, we explored effect measure modification by sex through stratification. We tested the significance of these interactions by including a delivery mode—pre-pregnancy BMI interaction term in the *Bacteroides* (the most abundant genus in our sample, which we previously showed to differ by delivery mode) differential abundance model[15].

Results

Study Population

The final sample for pre-pregnancy-BMI analyses comprised 335 mother-infant pairs, and 312 pairs for gestational-weight-gain analyses. Women in our study sample had a mean pre-pregnancy BMI of 25.9 kg/m^2 (SD: 5.5), with 55.5% classifying as normal weight, 26.9% as overweight, and 17.6% as obese. The mean gestational weight gain was 31.7 lbs (SD: 12.4), with 9.6% of the cohort experiencing inadequate weight gain, 27.8% adequate weight gain, and 55.8% excess weight gain. The majority of women in this cohort delivered vaginally (71.9%), exclusively breastfed their infants up to 6 weeks (69.0%), had a college degree (75.4%), and were Caucasian (99.4%).

In **Table 1,** we described mother-infant characteristics, jointly stratified by delivery mode and pre-pregnancy BMI categories. Among women who delivered vaginally, women with higher pre-pregnancy BMI tended to be younger, and were more likely to be primiparas (or to be delivering their first child), to have ever smoked, to have less education, and to have lower Mediterranean diet scores. Across both strata, we found that women with higher pre-pregnancy BMI had lower gestational weight gain, were less likely to exclusively breastfeed, had more male children, and were more likely to experience gestational hypertension. We show mother-infant characteristics jointly stratified by delivery mode and gestational weight gain in **Table S1.**

Overall Microbial Community Composition Structure (Beta Diversity)

Using unweighted UniFrac distances, we found that the overall microbial community composition structure of the 6-week stools was significantly different by pre-pregnancy BMI category in the vaginally delivered strata (PERMANOVA $p = 0.005$; **Figure S2**). However, there

was no evidence of clustering by pre-pregnancy BMI in Cesarean-born infants ($p = 0.135$, **Figure S3**), nor by gestational weight gain in vaginal or Cesarean-delivered infants ($p = 0.407$ & 0.701, **Figures S4 & S5**). Inter and intragroup distances are displayed in **Figure S6**. Similar trends were found for Bray-Curtis distances ($p = 0.139$ in vaginally delivered infants, and $p = 0.794$ in Cesarean delivered infants). With weighted UniFrac distances, pre-pregnancy BMI was significantly associated with beta diversity in Cesarean born infants ($p = 0.002$), but not in vaginally born infants ($p = 0.393$) All of these relationships were driven by the relationship between the obese group and the other two pre-pregnancy BMI groups. Results were consistent before and after adjustment for confounders in the vegan package.

Gut Microbiota Alpha Diversity

Alpha diversity indices are used to evaluate species richness (the number of unique OTUs) within a microbiome, as well as how evenly distributed they are within the ecosystem (evenness is taken into account by the Shannon diversity and Chao1 indices). Among vaginally-born infants, those with obese mothers had significantly greater alpha diversity (higher observed OTUs, $p = 0.012$), when compared to their normal weight counterparts, even after multivariable adjustment (**Figure 1**). Similar trends were found when alpha diversity was measured by the Shannon diversity index ($p = 0.069$) and Chao1 ($p = 0.010$) **(Figure S7)**. There were no significant findings between pre-pregnancy BMI and infant gut microbiota alpha diversity in infants delivered by Cesarean section (**Figure 1**; **Figure S7**). Moreover, gestational weight gain was not found to be independently associated with microbial alpha diversity in either vaginally-delivered or Cesarean-delivered infants **(Figure S8)**.

Differential Abundance of Gut Microbiota

Figure 2 shows the adjusted relative abundances by pre-pregnancy BMI across delivery mode strata, with infants born to normal weight mothers as the referent group. There was a borderline significant interaction between delivery mode and pre-pregnancy BMI on our *a priori* hypothesized genus of interest, *Bacteroides* (p for interaction = 0.05). There were opposing, non-significant trends with pre-pregnancy BMI in each delivery-mode stratum (**Figure 2**). In vaginally delivered infants, obesity was associated with greater abundance of both *Parabacteroides* ($p = 0.03$) and *Staphylococcus* ($p = 0.022$), which followed a graded linear relationship. Furthermore, among vaginally born neonates, we found higher abundance of *Escherichia* ($p < 0.001$), *Enterococcus* ($p = 0.017$), *Klebsiella* ($p = 0.037$), and *Ruminococcus* ($p < 0.001$) in infants born to overweight mothers, and decreased *Dorea* ($p = 0.043$) in infants born to obese mothers. Among infants delivered via Cesarean section, we also found obesity to be related to higher levels of *Staphylococcus* ($p = 0.022$), and lower levels of *Escherichia* ($p = 0.008$).

Gestational weight gain results are shown in **Figure S9**, with adequate weight gain as the referent group. Among vaginally-born infants, excessive weight gain was related to higher abundances of *Escherichia* ($p = 0.033$) and *Dorea* ($p < 0.001$). Within Cesarean-born infants, excessive weight gain was related to lower levels of *Dorea* ($p = 0.027$) and inadequate weight gain was related to lower levels of *Bifidobacterium* ($p = 0.027$).

To agnostically test for associations with taxa classification lower than the genus-level, we analyzed the differential abundance of bacterial OTUs, again according to pre-pregnancy BMI and gestational weight gain categories. Significant associations were *only* found in vaginally delivered infants. Infants born vaginally to overweight mothers, compared to normal

weight mothers, had significantly higher abundance of OTUs in the genera *Staphylcoccus* and *Enterococcus*, as well as species V. *dispar*, *B. fragilis*, and *E. coli* (FDR adjusted $p < 0.1$) (**Figure 3**). There were no significant results found for pre-pregnancy BMI in the Cesarean delivered arm, nor by gestational weight gain categories in vaginally or cesarean born infants.

Sensitivity Analyses

Our findings were not materially altered in sensitivity analyses in which we adjusted for feeding method at 6 weeks and or those in which we adjusted for sex. They were also similar when we examined associations in exclusively breastfed infants and conducted analyses separately in males and females.

Discussion

In vaginally born infants, pre-pregnancy BMI was associated with differences in most measures of infant gut microbial community structure (Unweighted Unifrac and Bray-Curtis, but not Weighted Unifrac) and diversity (richness and evenness), with infants born vaginally to obese mothers specifically having higher diversity. Among infants delivered vaginally, those born to overweight mothers also had higher abundance of 15 OTUs compared to those born to normal weight mothers. The association between pre-pregnancy BMI and several dominant taxa, including *Bacteroides*, the most abundant genus in the infant microbiome, varied significantly (p=0.05) by delivery mode, with trends in opposing directions. Pre-pregnancy BMI was not associated with microbial community diversity or differential taxa abundance in Cesarean-delivered infants. As such, our study provides further evidence that the association between maternal pre-pregnancy BMI and the infant microbiome is modified by delivery mode. We hypothesize the difference in associations by delivery mode may derive from whether an infant is exposed to maternal microbiota at birth.

Although previous studies have been conducted on the relationships between gestational weight gain, pre-pregnancy BMI and the infant gut microbiome, results thus far have been conflicting. The first study of this nature was conducted in 2010 by Collado et al. in a small Finnish cohort of 42 mothers[38]. In this study, infants born to overweight mothers had lower levels of *Bacteroides* at 1 month, and higher *Staphylococcus* at 6 months. Furthermore, they reported lower *Bacteroides* in the excess weight gain group when infants were 1 month, along with higher *Staphylococcus aureus* when they were 6 months. In our study, we found a similar association between pre-pregnancy BMI and *Staphylococcus*, but the direction of the association

of pre-pregnancy BMI with infant *Bacteroides* varied by delivery mode and was not significant. In another study, Galley and colleagues, in 77 mothers and their infants (18—27 months) from the US, reported that maternal obesity was associated with infant gut microbial community structure (beta diversity), (greater) diversity, and higher relative abundance of *Parabacteroides*, a similar finding to our study. However, some of these associations were restricted to their high socioeconomic strata[39]. A more recent study of 169 maternal-infant pairs from Norway reported no associations between pre-pregnancy BMI and the infant gut microbial structure in the first two years of life, but, similar to our study, they reported odds of having a higher prevalence of *Parabacteroides* in infants born to overweight or obese mothers[40].

Only two prior studies to our knowledge have assessed associations stratified by delivery mode. In 74 Brazilian mother-infant pairs[15], Mueller et al. found that pre-pregnancy BMI was associated with microbial community structure, but not alpha diversity, in the vaginal delivery strata, which was primarily driven by higher abundance of the genus *Bacteroides* in the overweight group. A more recent study by Tun et al. studied these relationships with the infant gut microbiome at 3–4 months in a large Canadian cohort of 935 mother-infant dyads[16]. In this study, the authors found that, similar to our study, maternal overweight/obese status was associated with greater infant microbial alpha diversity. Furthermore, linear discriminatory analyses revealed that in contrast to our findings, infants born vaginally to overweight/obese mothers had increased levels of the genus *Bacteroides*. The authors also reported positive associations with the Family *Lachnospiraceae*, which we did not observe in our study. Differences in timing of stool collection (6 weeks in our study vs. 3 – 4 months of age in Tun et al.), DNA extraction, primers used or sequencing may, in part, explain our divergent findings with respect to relative abundance of taxa.

As noted, our finding of higher bacterial (alpha) diversity among infants born vaginally to obese mothers is consistent with two previous studies[16,39]. Galley et al. reported higher alpha diversity in infants born to overweight/obese mothers compared to normal weight mothers, but these findings were restricted to their high socioeconomic status strata[39]. Tun and colleagues also found that maternal overweight/obese status was associated with higher alpha diversity in both vaginally-delivered and Cesarean-delivered infants at 3 – 4 months of age[16]. We only observed a significant association between pre-pregnancy BMI and infant microbiome diversity in the vaginally-delivered arm, although our Cesarean-born infants followed a similar but less marked trend, suggesting this may not only be due to differential sharing of microbiota at birth.

Little is known about the drivers of infant gut microbial alpha diversity within the first months of life, nor the association of early gut microbiome diversity and childhood health status. Azad and colleagues found that formula feeding compared to breastfeeding was associated with higher alpha diversity at 3 months of life, yet lower alpha diversity (in the same infants) at 1 year[41]. Our alpha findings were robust when we restricted to exclusively breastfed infants or when we adjusted for breastfeeding in multivariable models. We hypothesize that unlike in adulthood, where higher diversity is associated with better host health, higher microbial diversity in early infancy may indicate less mature immune system development. Large longitudinal birth cohort studies are needed to robustly examine the determinants of infant diversity in early life (first 3 months), its bi-directional relationship with immune development, and its association with disease risk prediction later in life.

Our finding of significantly higher *B. fragilis* in infants born vaginally to overweight mothers is worth highlighting as this particular species has been shown to be transferred from mother to newborn at birth [18], and it has been implicated in the development of childhood

overweight and obesity. Using culture-based techniques, a Belgian study of 138 infants found *B. fragilis* in infants (at 1 and 3 months age) is associated with higher BMI in later childhood[42]. Furthermore, a study of 84 Brazilian children 3 – 11 years old, along with a Netherlands cohort of 909 one-month old infants with BMI-z-scores measured between 1 and 10 years of age, showed similar results[43,44]. Further studies on this species and its function are warranted to understand its potential relationship with obesity and other metabolic diseases later in life.

We also reported graded linear associations of pre-pregnancy BMI with *Parabacteroides,* within vaginally delivered neonates, and with the genus *Staphylococcus* across both strata of delivery mode. OTUs within both of these genera were also significantly higher among infants born vaginally to overweight mothers. Considering *Parabacteroides* and *Staphylococcus* have found to be related to negative health outcomes in both children[45-48] and adults[49-53], future research is needed to assess if the associations between maternal weight status and these bacterial taxa mediate the risk for dysfunctional health, especially obesity and other cardiometabolic diseases, later in life.

In general, the gut microbiome of infants born vaginally to overweight and obese mothers showed a higher abundance of OTUs that are generally associated with gut dysbiosis (e.g. *B. Fragilis*, *E. coli*, *Enterococcus*, and *Staphylococcus*). Although there were several genera associated with gestational weight gain, on the whole associations of gestational weight gain with infant gut microbiome signatures were less marked, and gestational weight gain was not significantly associated with measures of diversity alpha diversity in vaginally-delivered or Cesarean-delivered infants. This finding suggests that pre-pregnancy BMI may be more influential than gestational weight gain on the development of the infant microbiome.

Our study has several strengths that distinguish it from previous studies in this field. The large sample size within the New Hampshire Birth Cohort Study allowed us to stratify by delivery mode, and to separate obese mothers from overweight mothers and inadequate weight gain from adequate weight gain, allowing us to evaluate graded, linear associations. Comprehensive data on covariates also enabled us to adjust for confounders of interest, and to test for effect measure modification in our models. However, there are also limitations to our study. The NHBC comprises a cohort of primarily Caucasian mother-infant pairs, which may not be representative of the general U.S. population. Also, because our analyses were restricted to stool samples at 6 weeks of age, our findings may not generalize to later developmental stages of infancy and childhood. Finally, our study is observational and thus we cannot rule out the possibility that unmeasured or residual confounding influenced our findings.

In conclusion, we found that pre-pregnancy BMI was associated with differential microbial community structure, diversity, and composition in the infant gut microbiome at 6 weeks of age. Yet, importantly, these associations were largely seen only in vaginally-delivered infants, consistent with the hypothesis that delivery mode may modify the mother-to-newborn transfer of maternal obese bacteria. Gestational weight gain was largely not associated with infant gut microbiome signatures in vaginally or Cesarean delivered infants, suggesting that pre-pregnancy BMI may be more influential than weight gain during gestation on the development of the infant microbiome. As the initial transfer of microbiota from mothers to their infants is crucial for providing the infant with bacteria to educate the immune system and to break down food to make energy, vitamins and minerals, there is clear need for continued study of potential perturbations to healthy mother-to-newborn transfer of microbiota and how these impacts may aid in understanding the early origins of various diseases, such as childhood obesity.

Furthermore, studies are necessary to replicate our results in other populations, to assess if these differences persist through childhood, and to determine how such differences may be related to differential risk for obesity, or other microbiome-related health outcomes later in life.

Tables

Table 1. Mother-infant characteristics jointly stratified by delivery mode and pre-pregnancy BMI category†

		Vaginally Delivered			Cesarean Section Delivered		
	Total (n = 335)	**Normal Weight** (n = 148)	**Overweight** (n = 58)	**Obese** (n = 35)	**Normal Weight** (n = 38)	**Overweight** (n = 32)	**Obese** (n = 24)
Maternal age, *years,* mean (SD)	32.0 (4.5)	32.2 (4.5)	31.2 (4.1)	30.6 (4.3)	33.2 (4.8)	31.7 (4.7)	32.9 (4.7)
Gestational age, *wk,* mean (SD)	39.5 (1.6)	39.5 (1.6)	39.88 (1.2)	39.4 (1.9)	39.3 (1.7)	39.2 (2.0)	38.9 (1.6)
Pre-pregnancy BMI, *kg/m2,* mean (SD)	25.9 (5.5)	22.2 (1.6)	26.9 (1.4)	35.1 (4.2)	22.3 (1.5)	27.3 (1.3)	36.1 (5.0)
Gestational weight gain, *lb,* mean (SD)	31.7 (12.4)	33.3 (10.5)	31.1 (12.8)	23.8 (13.6)	37.8 (14.8)	30.3 (9.5)	25.6 (12.9)
Gestational weight gain category, n (%)							
Inadequate weight gain	32 (9.6%)	16 (10.8%)	5 (8.6%)	4 (11.4%)	2 (5.3%)	2 (6.2%)	3 (12.5%)
Adequate weight gain	93 (27.8%)	57 (38.5%)	12 (20.7%)	9 (25.7%)	10 (26.3%)	3 (9.4%)	2 (8.3%)
Excess weight gain	187 (55.8%)	64 (43.2%)	39 (67.2%)	17 (48.6%)	26 (68.4%)	23 (71.9%)	18 (75.0%)
Missing	23 (6.9%)	11 (7.4%)	2 (3.4%)	5 (14.3%)	0 (0%)	4 (12.5%)	1 (4.2%)
Feeding at 6 weeks, n (%)							
Exclusively breastfed	231 (69.0%)	113 (76.4%)	40 (69.0%)	19 (54.3%)	28 (73.7%)	19 (59.4%)	12 (50.0%)
Combination feeding	60 (17.9%)	17 (11.5%)	11 (19.0%)	11 (31.4%)	5 (13.2%)	9 (28.1%)	7 (29.2%)
Exclusively formula fed	18 (5.4%)	7 (4.7%)	2 (3.4%)	2 (5.7%)	2 (5.3%)	2 (6.2%)	3 (12.5%)
Missing	23 (6.9%)	11 (7.4%)	5 (8.6%)	3 (8.6%)	3 (7.9%)	2 (6.2%)	2 (8.3%)
Infant sex, *fem,* n (%)	177 (52.8%)	74 (50.0%)	32 (55.2%)	14 (40.0%)	28 (73.7%)	17 (53.1%)	12 (50.0%)
Caucasian, n (%)	333 (99.4%)	148 (100.0%)	57 (98.3%)	35 (100.0%)	37 (97.4%)	32 (100.0%)	24 (100.0%)

Infant birth weight, g (SD)	3429.5 (508.4)	3418.0 (513.6)	3575.2 (414.8)	3268.5 (513.6)	3411.2 (556.2)	3421.1 (476.5)	3432.0 (591.1)
Birth weight/gestational age z-score, mean (SD)	0 (1.0)	0 (1.0)	0.3 (0.9)	-0.4 (0.8)	0.1 (1.2)	0 (1.2)	0 (1.1)
Parity, n (%)							
0	151 (45.1%)	57 (38.5%)	22 (37.9%)	19 (54.3%)	22 (57.9%)	17 (53.1%)	14 (58.3%)
1	132 (39.4%)	66 (44.6%)	27 (46.6%)	8 (22.9%)	13 (34.2%)	10 (31.2%)	8 (33.3%)
>= 2	52 (15.5%)	25 (16.9%)	9 (15.5%)	8 (22.9%)	3 (7.9%)	5 (15.6%)	2 (8.3%)
Gestational diabetes, n (%)	25 (8.0%)	6 (4.3%)	6 (10.9%)	1 (3.1%)	1 (2.7%)	7 (25.0%)	4 (17.4%)
Pre-eclampsia, n (%)	18 (5.7%)	7 (5.0%)	0 (0%)	2 (6.2%)	3 (8.1%)	3 (10.7%)	3 (13.0%)
Gestational hypertension, n (%)	30 (9.5%)	7 (5.0%)	4 (7.3%)	6 (18.2%)	2 (5.4%)	4 (14.3%)	7 (30.4%)
Highest education achieved, n (%)							
High School or less	29 (9.0%)	12 (8.4%)	5 (9.1%)	4 (12.9%)	4 (10.5%)	2 (6.5%)	2 (8.7%)
Some college	50 (15.6%)	19 (13.3%)	14 (25.5%)	7 (22.6%)	5 (13.2%)	3 (9.7%)	2 (8.7%)
College degree	120 (37.4%)	51 (35.7%)	20 (36.4%)	9 (29.0%)	13 (34.2%)	16 (51.6%)	11 (47.8%)
Graduate school	122 (38.0%)	61 (42.7%)	16 (29.1%)	11 (35.5%)	16 (42.1%)	10 (32.3%)	8 (34.8%)
Mediterranean Diet Score, mean (SD)	3.8 (1.8)	4.15 (1.70)	3.46 (2.08)	2.87 (1.76)	3.92 (1.92)	3.32 (1.49)	3.96 (1.77)
SSB consumption, n (%)							
None	93 (27.8%)	38 (25.7%)	19 (32.8%)	9 (25.7%)	12 (31.6%)	11 (34.4%)	4 (16.7%)
1/month - 3/month	62 (18.5%)	34 (23.0%)	5 (8.6%)	6 (17.1%)	9 (23.7%)	5 (15.6%)	3 (12.5%)
1/week - 6/week	135 (40.3%)	60 (40.5%)	26 (44.8%)	11 (31.4%)	15 (39.5%)	10 (31.2%)	13 (54.2%)
1/day or more	45 (13.4%)	16 (10.8%)	8 (13.8%)	9 (25.7%)	2 (5.3%)	6 (18.8%)	4 (16.7%)
Low calorie beverage							

consumption, n (%)							
None	230 (68.7%)	104 (70.3%)	39 (67.2%)	31 (88.6%)	29 (76.3%)	16 (50.0%)	11 (45.8%)
1/month - 3/month	33 (9.9%)	20 (13.5%)	7 (12.1%)	1 (2.9%)	2 (5.3%)	3 (9.4%)	0 (0%)
1/week - 6/week	58 (17.3%)	19 (12.8%)	11 (19.0%)	3 (8.6%)	7 (18.4%)	11 (34.4%)	7 (29.2%)
1/day or more	14 (4.2%)	5 (3.4%)	1 (1.7%)	0 (0%)	0 (0%)	2 (6.2%)	6 (25.0%)
Smoking, n (%)							
Ever smoked	36 (11.1%)	126 (88.1%)	50 (90.9%)	27 (81.8%)	34 (89.5%)	29 (93.5%)	21 (91.3%)
Smoked during pregnancy	20 (6.2%)	9 (6.3%)	3 (5.5%)	6 (18.2%)	0 (0%)	1 (3.2%)	1 (4.3%)
Never smoked	287 (88.9%)	8 (5.6%)	2 (3.6%)	0 (0%)	4 (10.5%)	1 (3.2%)	1 (4.3%)
Alcohol, *drinks/wk,* mean (SD)	3.5 (4.8)	3.7 (5.2)	3.1 (4.5)	2.3 (4.4)	3.5 (2.9)	5.4 (5.8)	2.0 (3.2)
Prenatal anbiotics, n (%)	51 (16.4%)	22 (15.9%)	8 (14.8%)	4 (12.9%)	6 (16.2%)	6 (21.4%)	5 (21.7%)
Age at formula introduction among combination fed subjects, wk (SD)	18.3 (16.3)	20.9 (17.3)	18.7 (16.8)	14.7 (14.2)	16.4 (13.7)	17.7 (17.0)	14.1 (15.7)
Gut microbiome sample age *wk,* mean (SD)	6.8 (2.6)	6.7 (2.0)	6.5 (1.1)	8.0 (4.8)	6.5 (1.0)	7.6 (4.4)	6.1 (0.9)

† The following variables had missing data that is not shown in the table: birthweight (n = 10), birthweight z score (n = 14), gestational diabetes (n = 21), Pre-eclampsia (n = 21), gestational hypertension (n = 20), education (n = 14), Mediterranean diet score (n = 17), smoking (n = 12), prenatal antibiotics (n = 24), age first formula (n = 113).

Supplemental Table S1. Mother-infant characteristics jointly stratified by delivery mode and gestational weight gain category[†]

		Vaginally Delivered			Cesarean Section Delivered		
	Total (n = 335)	**Inadequate Weight Gain** (n = 25)	**Adequate Weight Gain** (n = 78)	**Excess Weight Gain** (n = 120)	**Inadequate Weight Gain** (n = 7)	**Adequate Weight Gain** (n = 15)	**Excess Weight Gain** (n = 67)
Maternal age, *years,* mean (SD)	32.2 (4.4)	33.8 (4.2)	32.3 (3.9)	31.5 (4.5)	34.0 (5.4)	33.1 (4.6)	32.3 (4.7)
Gestational age, *wk,* mean (SD)	39.5 (1.6)	39.7 (1.2)	39.7 (1.5)	39.5 (1.6)	39.2 (1.4)	38.9 (2.7)	39.2 (1.6)
Pre-pregnancy BMI, *kg/m2,* mean (SD)	25.7 (5.3)	25.7 (5.2)	24.4 (4.5)	25.3 (4.8)	30.8 (8.9)	25.4 (7.2)	27.5 (5.6)
Gestational weight gain, *lb,* mean (SD)	31.7 (12.4)	14.3 (7.1)	25.7 (4.9)	38.8 (10.1)	10.9 (7.5)	22.7 (5.4)	36.7 (12.2)
BMI category, n (%)							
Normal weight	175 (56.1%)	16 (64.0%)	57 (73.1%)	64 (53.3%)	2 (28.6%)	10 (66.7%)	26 (38.8%)
Overweight	84 (26.9%)	5 (20.0%)	12 (15.4%)	39 (32.5%)	2 (28.6%)	3 (20.0%)	23 (34.3%)
Obese	44 (14.1%)	4 (16.0%)	9 (11.5%)	17 (14.2%)	3 (42.9%)	2 (13.3%)	18 (26.9%)
Feeding at 6 weeks, n (%)							
Exclusively breastfed	219 (70.2%)	16 (64.0%)	60 (76.9%)	86 (71.7%)	3 (42.9%)	13 (86.7%)	41 (61.2%)
Combination feeding	55 (17.6%)	5 (20.0%)	11 (14.1%)	19 (15.8%)	3 (42.9%)	1 (6.7%)	16 (23.9%)
Exclusively formula fed	15 (4.8%)	1 (4.0%)	2 (2.6%)	6 (5.0%)	1 (14.3%)	1 (6.7%)	4 (6.0%)
Missing	23 (7.4%)	3 (12.0%)	5 (6.4%)	9 (7.5%)	0 (0%)	0 (0%)	6 (9.0%)
Infant sex, *fem,* n (%)	167 (53.5%)	12 (48.0%)	41 (52.6%)	59 (49.2%)	2 (28.6%)	9 (60.0%)	44 (65.7%)
Caucasian, n (%)	310 (99.4%)	24 (96.0%)	78 (100.0%)	120 (100.0%)	7 (100.0%)	15 (100.0%)	66 (98.5%)
Infant birth weight, g (SD)	3449.6 (503.0)	3548.1 (469.2)	3453.7 (450.9)	3442.9 (512.3)	3081.4 (363.1)	3381.0 (570.6)	3476.7 (547.6)
Birth weight/gestational age z-score, mean (SD)	0.1 (1.0)	0 (0.7)	0 (1.0)	0.1 (1.0)	-0.8 (0.6)	-0.1 (1.3)	0.2 (1.2)
Parity, n (%)							
0	139 (44.6%)	7 (28.0%)	32 (41.0%)	49 (40.8%)	3 (42.9%)	10 (66.7%)	38 (56.7%)
1	125 (40.1%)	13 (52.0%)	32 (41.0%)	51 (42.5%)	4 (57.1%)	4 (26.7%)	21 (31.3%)
>= 2	48 (15.3%)	5 (20.0%)	14 (17.9%)	20 (16.7%)	0 (0%)	1 (6.7%)	8 (11.9%)
Gestational diabetes, n (%)	25 (8.1%)	1 (4.0%)	8 (10.3%)	4 (3.4%)	3 (42.9%)	1 (6.7%)	8 (12.1%)
Pre-eclampsia, n (%)	18 (5.8%)	0 (0%)	0 (0%)	9 (7.7%)	2 (28.6%)	0 (0%)	7 (10.6%)
Gestational hypertension, n (%)	29 (9.4%)	0 (0%)	2 (2.6%)	14 (11.9%)	1 (14.3%)	1 (6.7%)	11 (16.7%)

Highest education achieved, n (%)							
High School or less	23 (7.5%)	0 (0%)	5 (6.7%)	10 (8.5%)	0 (0%)	1 (6.7%)	7 (10.4%)
Some college	46 (15.0%)	4 (16.0%)	8 (10.7%)	24 (20.3%)	2 (28.6%)	0 (0%)	8 (11.9%)
College degree	118 (38.4%)	12 (48.0%)	22 (29.3%)	46 (39.0%)	3 (42.9%)	3 (20.0%)	32 (47.8%)
Graduate school	120 (39.1%)	9 (36.0%)	40 (53.3%)	38 (32.2%)	2 (28.6%)	11 (73.3%)	20 (29.9%)
Mediterranean Diet Score, mean (SD)	3.8 (1.8)	4.3 (1.9)	3.9 (1.7)	3.7 (1.9)	4.4 (2.0)	4.0 (1.7)	3.6 (1.8)
SSB consumption, n (%)							
None	80 (25.6%)	5 (20.0%)	21 (26.9%)	30 (25.0%)	2 (28.6%)	5 (33.3%)	17 (25.4%)
1/month - 3/month	59 (18.9%)	5 (20.0%)	15 (19.2%)	23 (19.2%)	1 (14.3%)	1 (6.7%)	14 (20.9%)
1/week - 6/week	130 (41.7%)	10 (40.0%)	35 (44.9%)	48 (40.0%)	2 (28.6%)	8 (53.3%)	27 (40.3%)
1/day or more	43 (13.8%)	5 (20.0%)	7 (9.0%)	19 (15.8%)	2 (28.6%)	1 (6.7%)	9 (13.4%)
Low calorie beverage consumption, n (%)							
None	209 (67.0%)	17 (68.0%)	52 (66.7%)	89 (74.2%)	4 (57.1%)	12 (80.0%)	35 (52.2%)
1/month - 3/month	33 (10.6%)	6 (24.0%)	10 (12.8%)	12 (10.0%)	0 (0%)	1 (6.7%)	4 (6.0%)
1/week - 6/week	57 (18.3%)	1 (4.0%)	14 (17.9%)	17 (14.2%)	2 (28.6%)	2 (13.3%)	21 (31.3%)
1/day or more	13 (4.2%)	1 (4.0%)	2 (2.6%)	2 (1.7%)	1 (14.3%)	0 (0%)	7 (10.4%)
Smoking, n (%)							
Ever smoked	30 (9.7%)	24 (96.0%)	68 (89.5%)	105 (89.0%)	7 (100.0%)	15 (100.0%)	59 (88.1%)
Smoked during pregnancy	14 (4.5%)	0 (0%)	5 (6.6%)	7 (5.9%)	0 (0%)	0 (0%)	2 (3.0%)
Never smoked	278 (90.3%)	1 (4.0%)	3 (3.9%)	6 (5.1%)	0 (0%)	0 (0%)	6 (9.0%)
Alcohol, *drinks/wk,* mean (SD)	3.5 (4.3)	4.3 (5.8)	3.9 (4.5)	2.9 (3.5)	3.9 (5.2)	2.6 (2.7)	4.2 (4.6)
Prenatal anbiotics, n (%)	51 (16.7%)	6 (24.0%)	11 (14.3%)	17 (14.7%)	2 (28.6%)	3 (21.4%)	12 (17.9%)
Age at formula introduction among combination fed subjects, wk (SD)	18.4 (16.3)	20.8 (18.0)	20.9 (17.7)	18.4 (16.3)	3.7 (5.8)	14.7 (7.5)	17.5 (16.2)
Gut microbiome sample age *wk,* mean (SD)	6.7 (2.4)	6.8 (1.8)	6.8 (2.7)	6.8 (2.5)	6.4 (1.6)	7.4 (4.1)	6.4 (1.7)

† Regarding missingness, the following variables had missing data that is not shown in the table: birthweight (n = 10), birthweight z score (n = 14), gestational diabetes (n = 4), Pre-eclampsia (n = 4), gestational hypertension (n = 3), education (n = 5), Mediterranean diet score (n = 7), smoking (n = 4), prenatal antibiotics (n = 6), age first formula (n = 106).

Figures

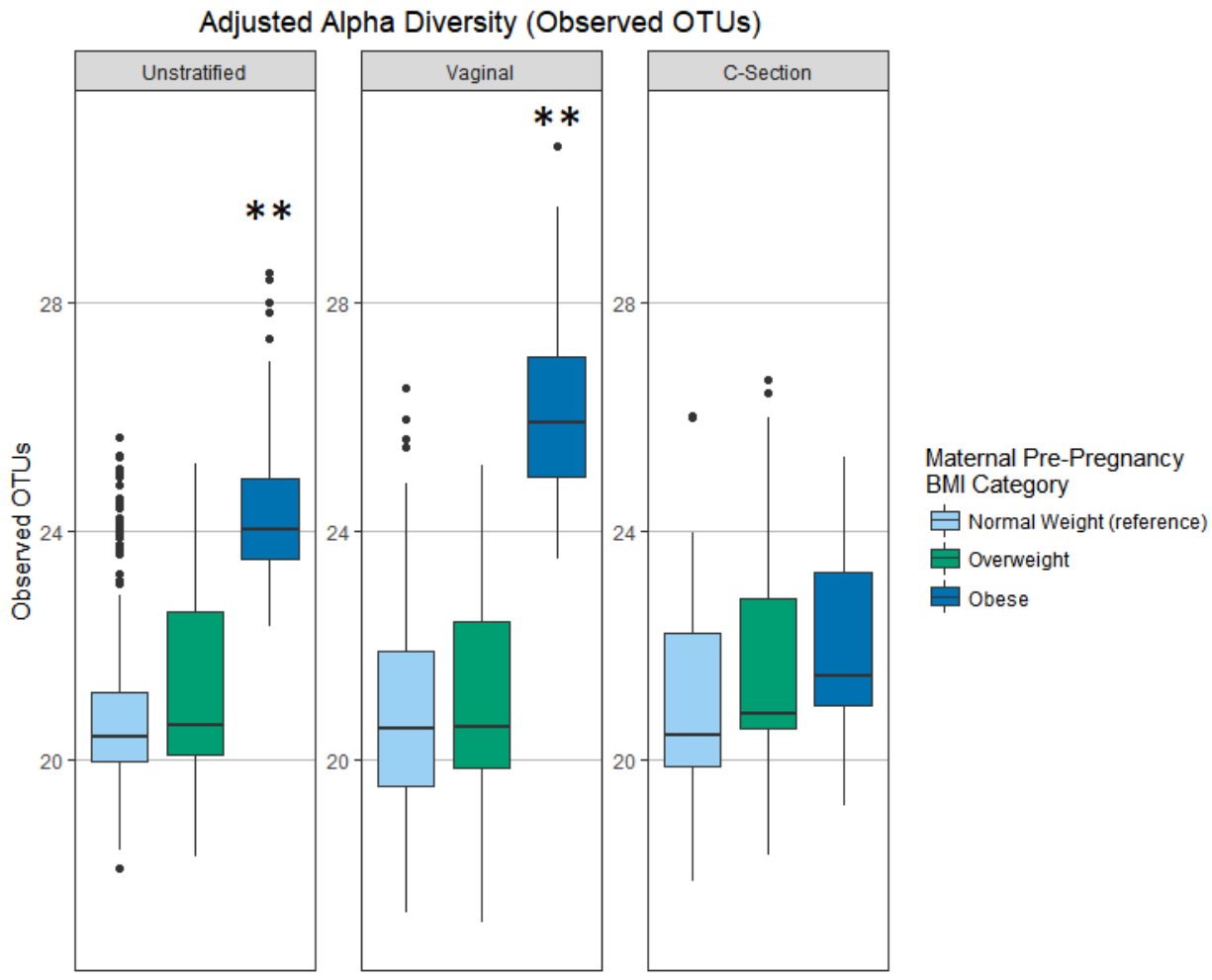
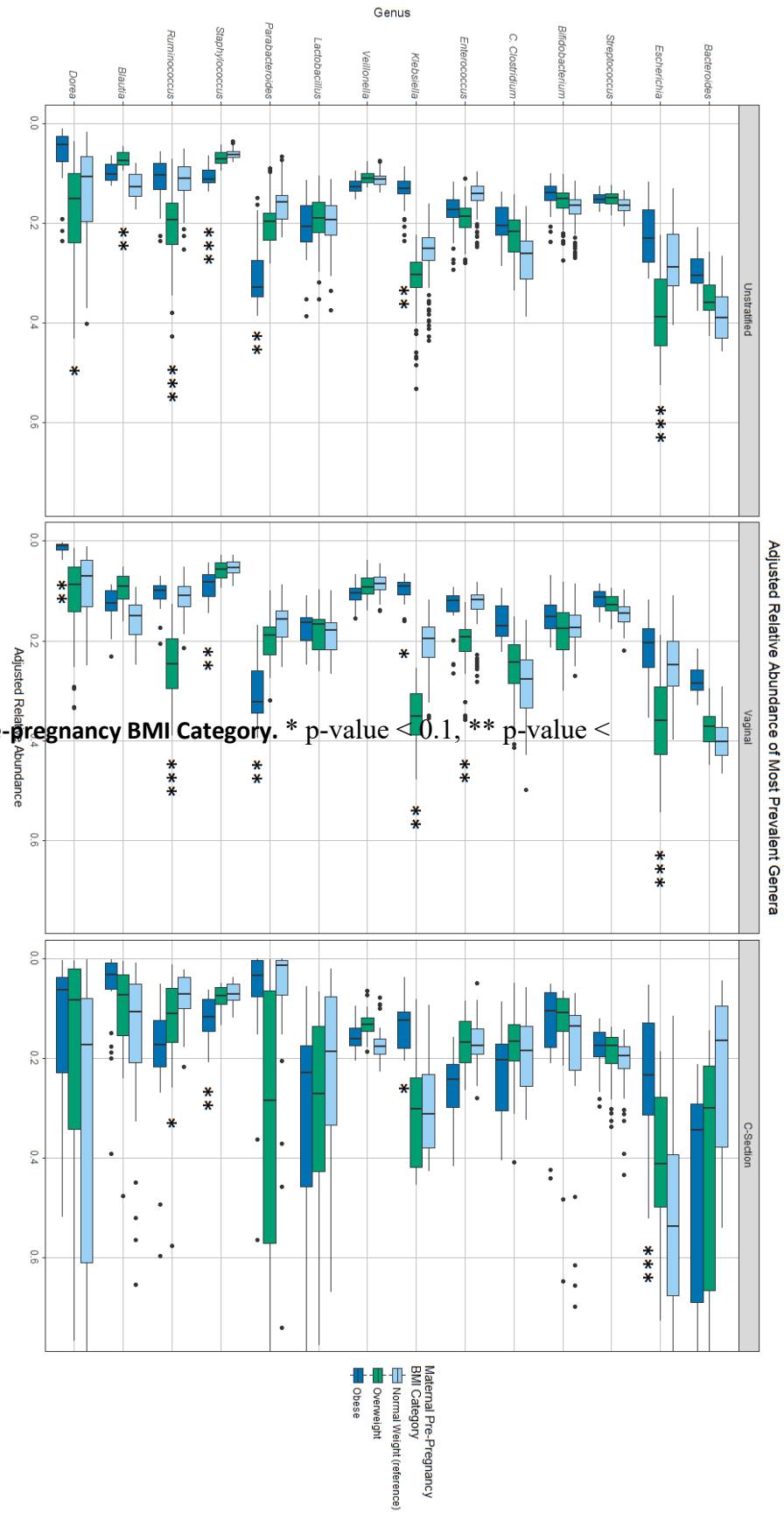


Figure 1. Adjusted infant gut microbial alpha-diversity by pre-pregnancy BMI category, measured by Observed OTUs. ** p-value < 0.05.



bundant genera (> 1%) by pre-pregnancy BMI Category. * p-value < 0.1, ** p-value <

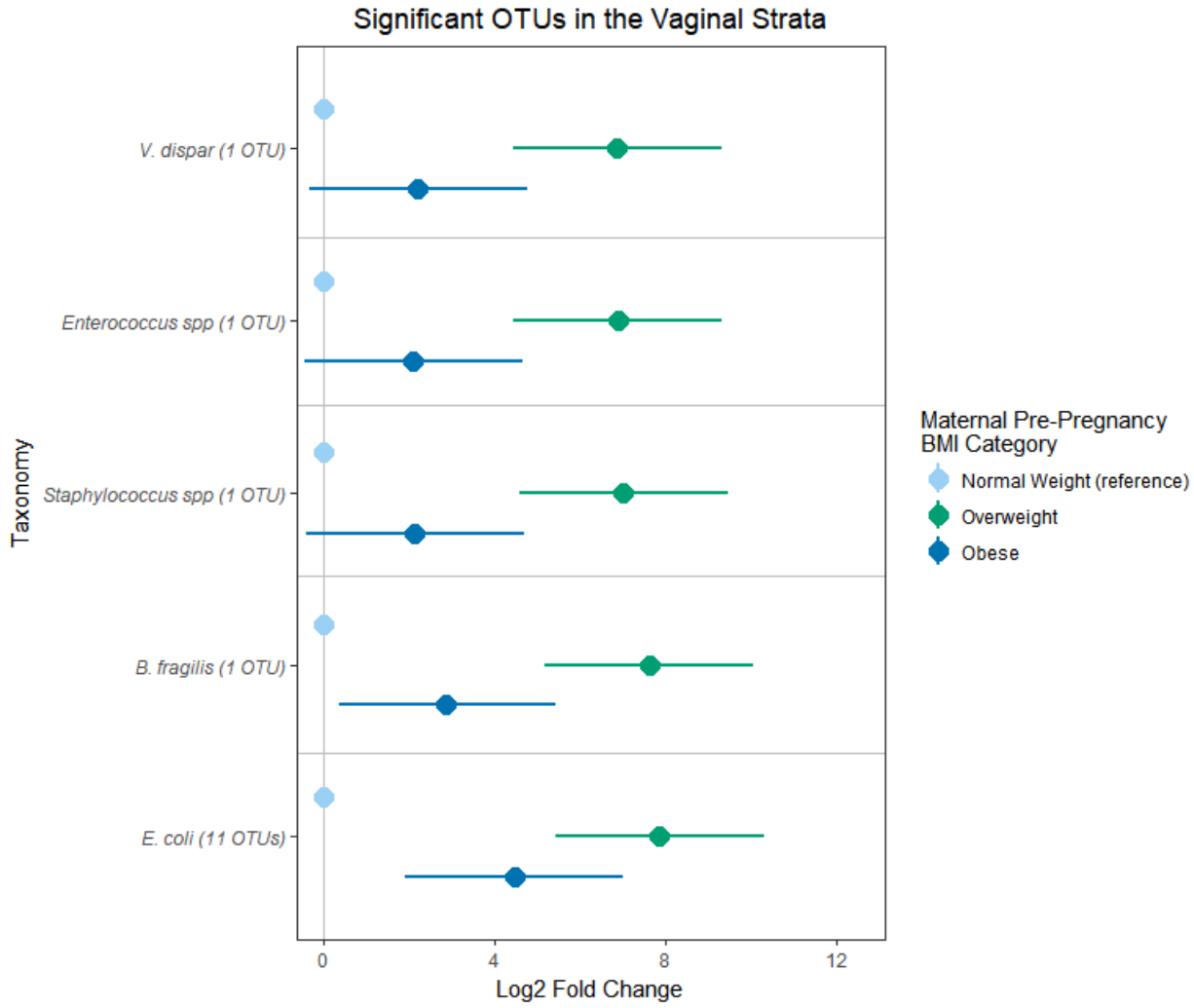
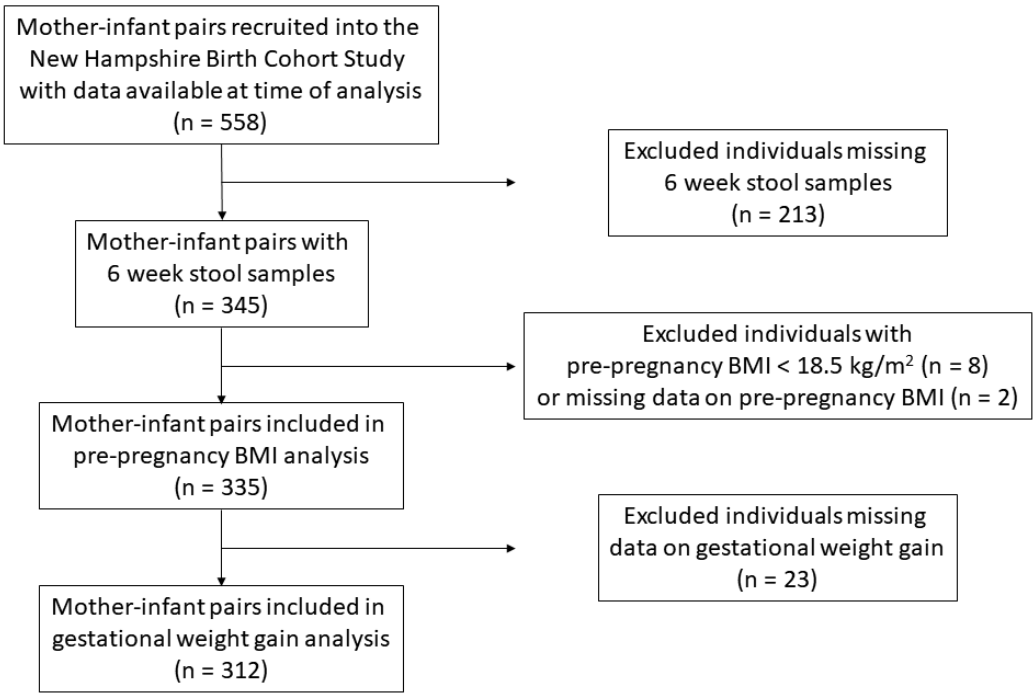
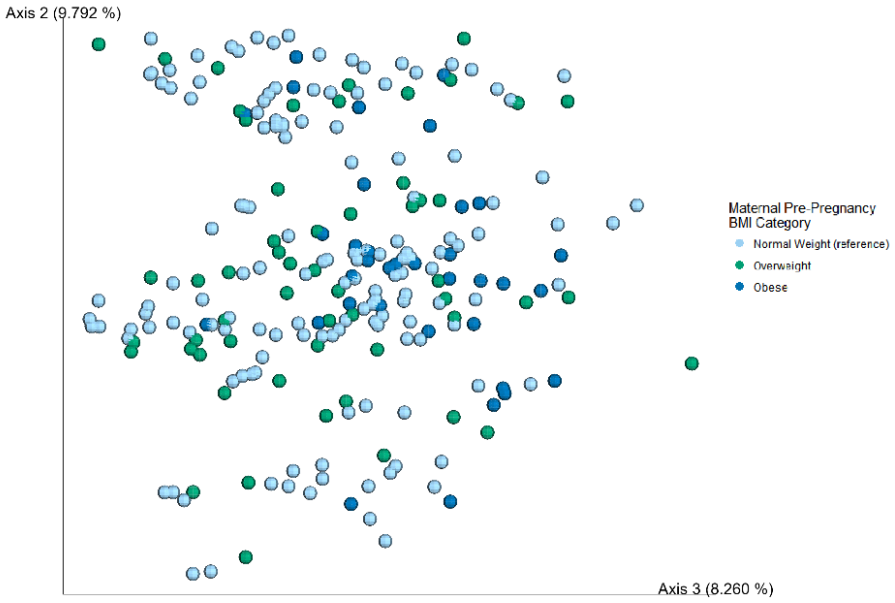


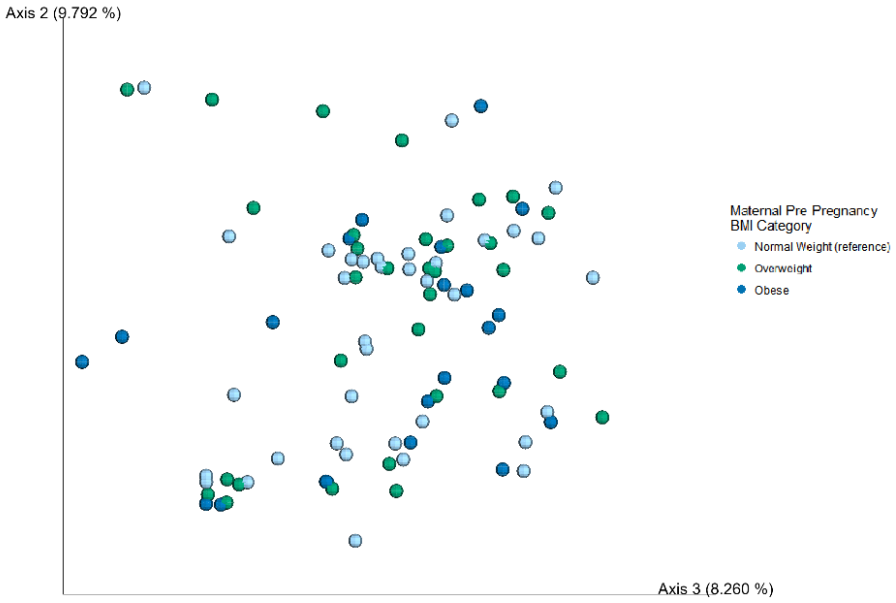
Figure 3. Significant OTUs that were significantly differentially abundant (FDR-adjusted p-value < 0.1). Only vaginally born infants with overweight mothers had significant differences in the abundance of OTUs, compared to normal weight mothers.



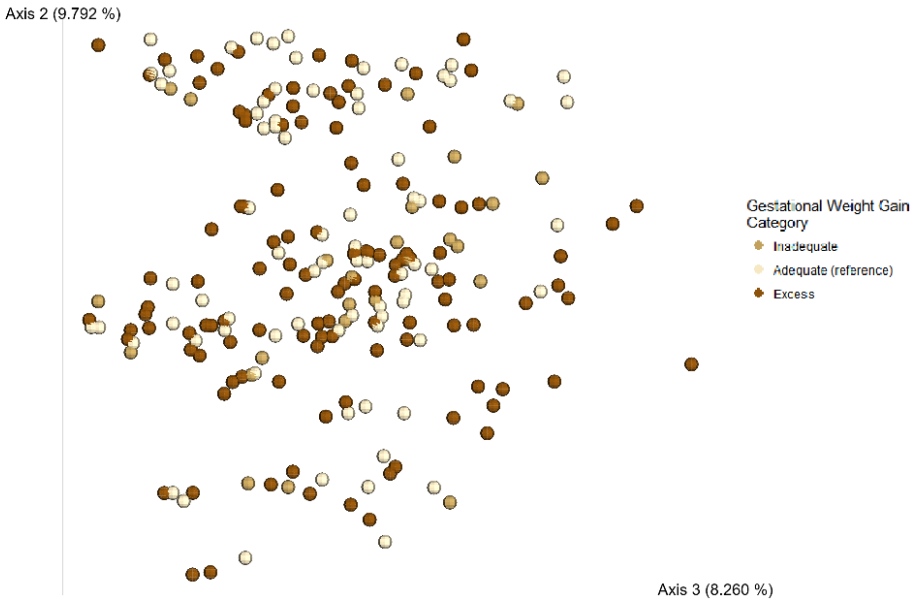
Supplemental Figure S1. Flowchart of the New Hampshire Birth Cohort mother-child pairs selected for our analytic sample.



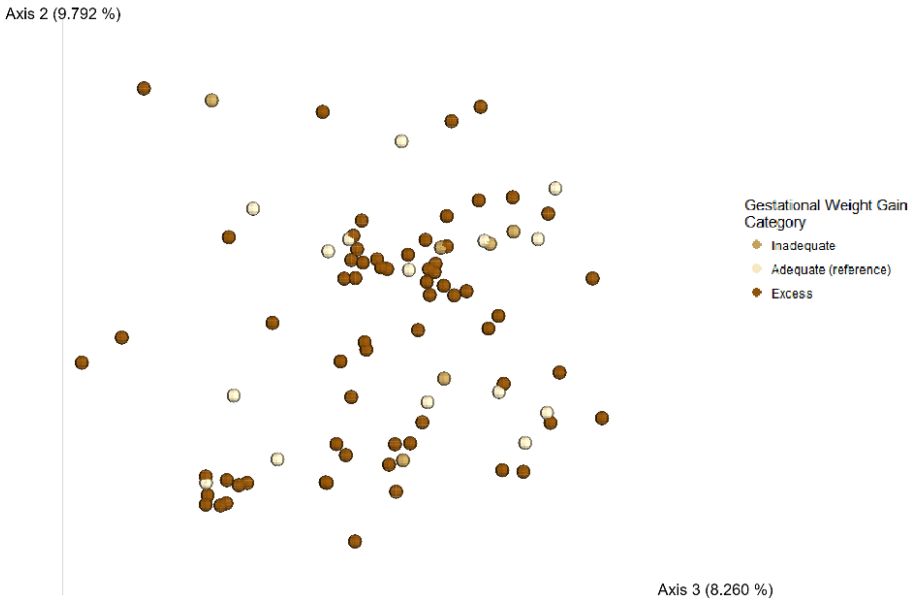
Supplemental Figure S2. Infant gut microbial beta diversity of vaginally born infants by pre-pregnancy BMI category using unweighted UniFrac distance. Axes 2 and 3 are displayed. Overall PERMANOVA p-value = 0.005.



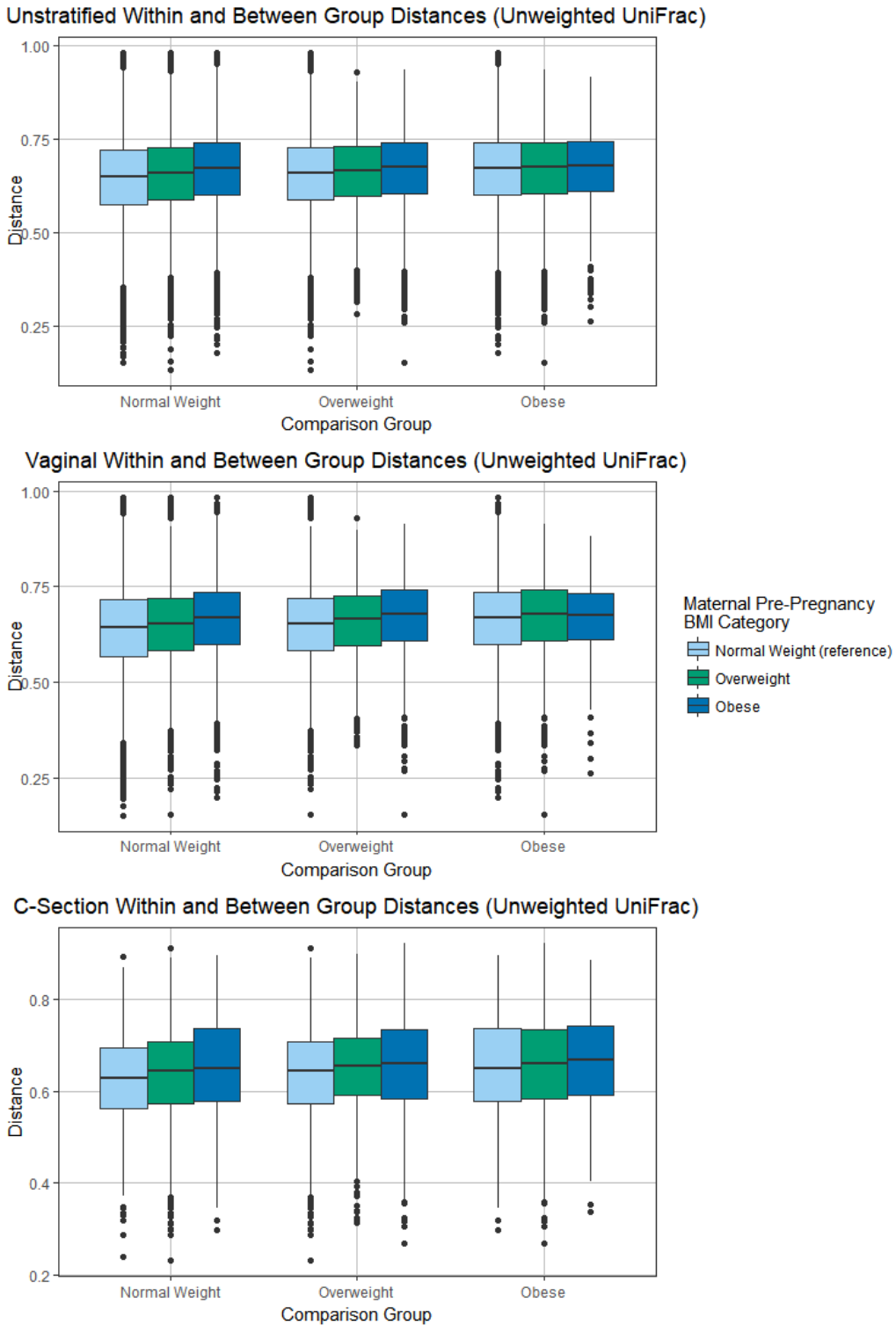
Supplemental Figure S3. Infant gut microbial beta diversity of Cesarean born infants by gestational weight gain category using unweighted UniFrac distance. Axes 2 and 3 are displayed. Overall PERMANOVA p-value = 0.135.



Supplemental Figure S4. Infant gut microbial beta diversity of vaginally born infants by gestational weight gain category using unweighted UniFrac distance. Axes 2 and 3 are displayed. Overall PERMANOVA p-value = 0.407.

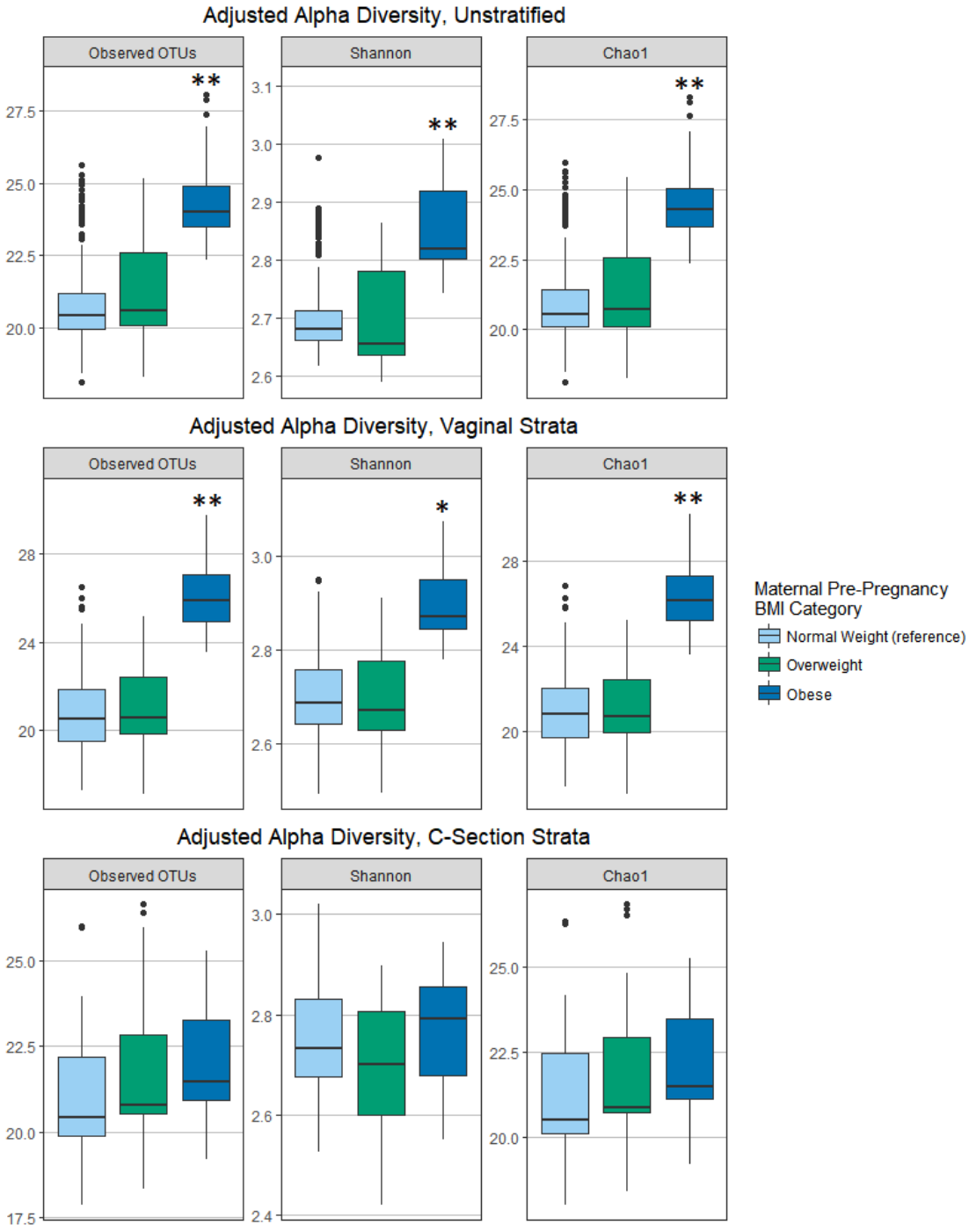


Supplemental Figure S5. Infant gut microbial beta diversity of Cesarean born infants by gestational weight gain category using unweighted UniFrac distance. Axes 2 and 3 are displayed. Overall PERMANOVA p-value = 0.701.

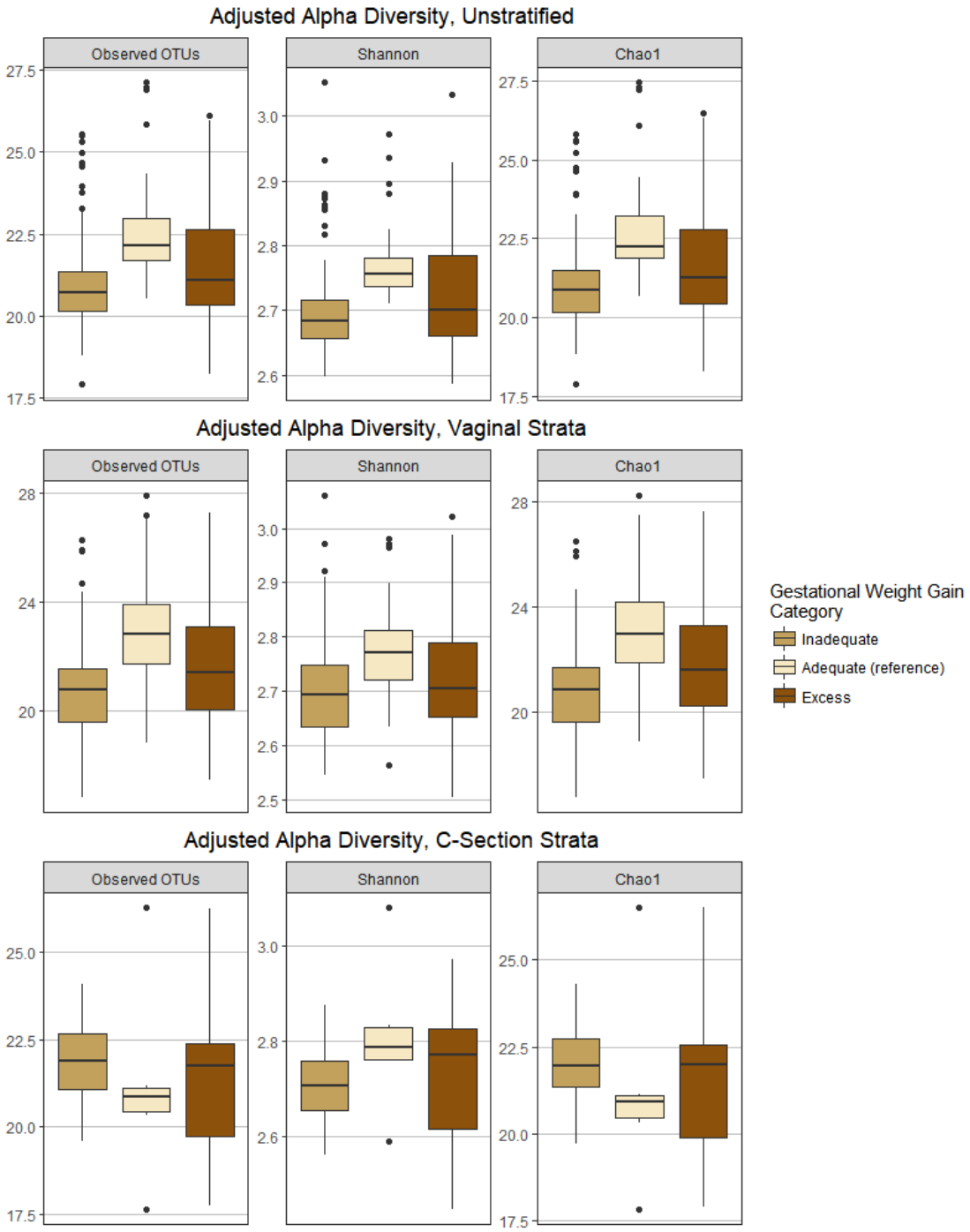


Supplemental Figure S6. Unadjusted within group and between group beta-diversity distances by pre-pregnancy BMI category using unweighted UniFrac distances, stratified

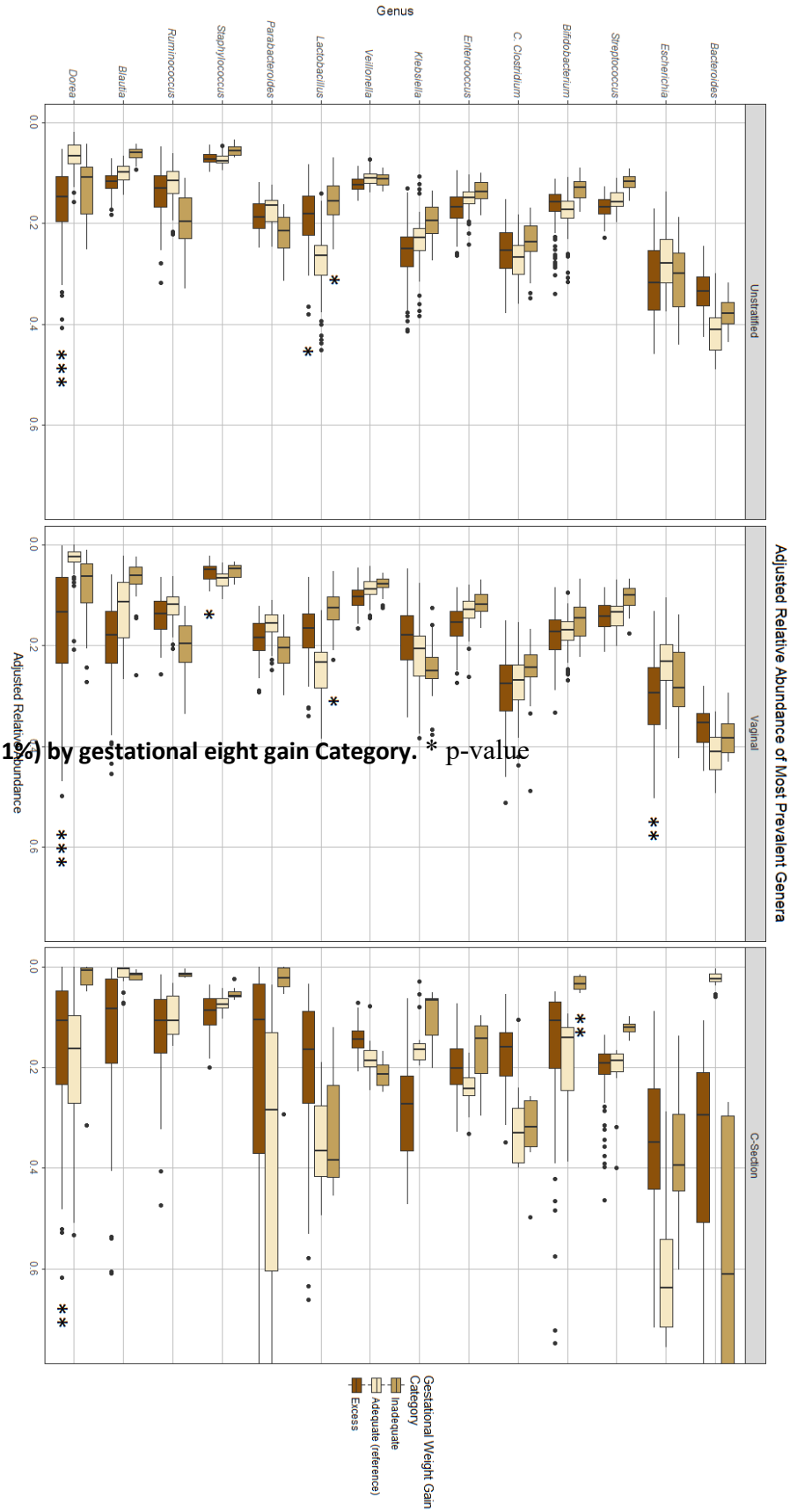
by delivery mode. X axis label for pre-pregnancy BMI group indicates the comparison group, for which within-group distances are displayed for that group. The remaining two boxplots in that group display the distances between the comparison group and the other two pre-pregnancy BMI groups. Pairwise PERMANOVA tests revealed that the obese group was significantly different than the normal weight group when taking into account both within-group and between group distances (q-values: unstratified = 0.006, vaginal = 0.018, C-section = 0.060). Furthermore, the overweight group was significantly different than the obese group in vaginally born infants (q-value = 0.027), leading to an overall significant relationship between pre-pregnancy BMI category and beta diversity in vaginally born infants (p-value = 0.005).



Supplemental Figure S7. Adjusted infant gut microbial alpha diversity by pre-pregnancy BMI category, stratified by delivery mode. Alpha diversity was measured using Observed OTUs, Shannon Diversity Index, and Chao1. * p-value < 0.1, ** p-value < 0.05.



Supplemental Figure S8. Adjusted infant gut microbial alpha diversity by gestational weight gain category, stratified by delivery mode. Alpha diversity was measured using Observed OTUs, Shannon Diversity Index, and Chao1.



nce of most abundant genera (> 1%) by gestational eight gain Category. * p-value

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Curriculum Vitae

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EDUCATION AND TRAINING

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AS (2012), Mathematics

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BA (2016), Public Health Studies (concentration in Nutrition)

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PROFESSIONAL EXPERIENCE

Research Assistant (06/2016 – Present)

Welch Center for Prevention, Epidemiology and Clinical Research

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Johns Hopkins Weight Management Center

Department of Health Behavior Change and Society

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Research Assistant (08/2016 – 05/2017)

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Intern (Summer of 2015)

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Research Assistant (Summers of 2013 and 2014)

Internal Medicine Division of Cardiovascular Medicine

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Research Assistant (2012-2013)

Department of Neuroscience

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Research Assistant (2008 – 2012)

School of Plant Sciences, School of Animal & Comparative Biomedical Sciences and College of Agriculture and Life Sciences

University of Arizona

AWARDS AND SCHOLARSHIPS

Nancy Fink Fund for Scholarship and Service in the Department of Epidemiology, 2017

PROFESSIONAL ACTIVITIES

Leadership and Outreach

Leader of Biweekly Meditation Sessions (01/2018 – 05/2018)

Johns Hopkins Eastern Campuses

Member, Sanctuary Streets (01/2018 – Present)

Johns Hopkins University

Member, Interfaith Council (2013 – Present)

Johns Hopkins University

President and Member, Sikh Student Association (2013 – Present)

Johns Hopkins University

Member, Hopkins Leadership Initiative for the Environment (2016 – present)

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Treasurer, Students for Environmental Action (2013 – 2016)

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Member, Refuel our Future (2013 – 2016)

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PUBLICATIONS

Journal Articles

1. **Singh S**, Karagas MR, Mueller NT. Charting the Maternal and Infant Microbiome: What Is the Role of Diabetes and Obesity in Pregnancy?. *Curr Diab Rep.* 2017 Feb;17(2):11. doi: 10.1007/s11892-017-0836-9. Review.

ADDITIONAL INFORMATION

Statement of Research: My research interests lie at the nexus of nutrition, epidemiology, and cardiometabolic disease prevention. I am pursuing an MHS in Epidemiology to obtain a skill set that will allow me to identify modifiable factors, such as diet and the microbiome, that can help to alleviate the large burden of obesity, type 2 diabetes and hypertension around the world; particularly in low- and middle-income countries such as India, where I am from. Recently, working with my mentor Dr. Noel Mueller, my interests have shifted towards the human gut microbiome as a site for intervention. Already as an MHS student I have had the opportunity to publish a review paper on the association of the microbiome with obesity and diabetes in pregnancy, and for my MHS thesis I plan to work with Dr. Mueller to examine the relationship between maternal metabolic dysfunction, and the infant gut microbiome in the New Hampshire Birth Cohort.

Key words: Obesity, Type 2 Diabetes, Cardiovascular Disease, Nutrition, Epidemiology, Microbiome